

REMARKS

Claims 108-146 are pending herein and claim 108 is amended. Applicants reserve the right to pursue subject matter in amended subject matter in a related patent application. Amended claim 108 is directed to commercial embodiments and adds no new matter as the specification provides basis, for example, on page 23, line 12 and page 26, line 35. Thus, entry of the claim amendment is proper. Reconsideration of rejections in the Office action is requested, as discussed below.

Rejection under 35 U.S.C. §112, second paragraph

Claims 144 and 145 were rejected under 35 U.S.C. §112, second paragraph, for alleged lack of clarity. The Office stated it is not clear as to which two dimensions are meant in defining the spot size. The rejection respectfully is traversed. The claims specify the spot size is defined by two dimensional parameters. The specification makes it clear that in some embodiments a solution was spotted in wells of a substrate having sides of defined length. The specification notes in certain embodiments the wells had an inverted flat top pyramidal shape (e.g., page 29, line 27; page 26, line 23 to page 27, line 8). As the claimed volumes spotted do not fill the entire void of such wells, the resulting spot is defined by the dimensions of the well openings (e.g., 800 x 800 micrometers, 450 x 450 micrometers). Thus, Applicants respectfully assert the subject matter of claims 144 and 145 is clear and withdrawal of the rejection is requested.

Obviousness Rejection: Combination of Vestal, Vorm and Hayes

Claims 108-118, 120-123, 125, 132-141 and 143-146 were rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious by Vestal in view of Vorm *et al.* and Hayes *et al.* The Office states Vestal teaches an apparatus for automated MALDI mass spectrometric (MS) analysis for a plurality of DNA samples combined with matrix. The Office acknowledges that Vestal does not teach depositing MALDI matrix without an analyte or allowing a spot of matrix to dry in the absence of an analyte. The Office interprets Vorm as teaching methods for depositing microliter volumes of matrix on a MALDI MS probe tip. While acknowledging that Vestal and Vorm do not teach depositing 0.2 to 20 nanoliter volumes, the Office concludes it would have been obvious to modify Vestal's method of MALDI MS analysis with Vorm's method of depositing MALDI

matrix, and that Hayes provides a teaching of dispensing the claimed volumes.

Claimed subject matter is *prima facie* obvious only when the cited document or combination of documents teaches or suggests all of the claimed elements, the person of ordinary skill in the art was motivated to modify the document(s) as suggested in the Office action, and there was a reasonable expectation of success. See MPEP 2142, et seq.

Applicants respectfully submit the rejection is moot with respect to amended independent claim 108 and its dependent claims. These claims are directed to methods for constructing substrates having an array of spots consisting essentially of a particular matrix, 3-HPA. The Declaration under 37 C.F.R. §1.132 by Thomas Becker makes it clear such methods and substrates are inventive over those discussed in the cited documents.

First, Vorm discusses substrates having particular matrix components for mass spectrometric analysis of proteins. Vorm therefore does not mention the matrix 3-HPA as it generally is utilized for mass spectrometric analysis of nucleic acids. Vestal and Hayes also fail to mention the matrix 3-HPA. The Declaration clarifies that properties of 3-HPA differ significantly from those of matrices utilized for protein analysis. For example, the Declaration states in paragraph 4:

The protein/peptide matrix components typically form small, homogeneous crystals when spotted on a substrate. This small crystal homogeneity facilitates accurate spot-to-spot mass spectra reproducibility. In contrast, 3-HPA applied under the conditions reported in the documents cited by the Office yields a crystal morphology that does not provide consistent mass spectra results. Spots resulting from deposition of analyte on the 3-HPA matrix spots pre-deposited under conditions of the cited documents often have amorphous structure. These amorphous structures often lead to poor mass spectra reproducibility.

The Declaration concludes the following in paragraph 5:

Research pertaining to protein/peptide matrix components was not applicable to developing the claimed substrates for mass spectrometric analysis due to the different 3-HPA crystal morphology (i.e., amorphous structure). It was not until the claimed substrates were generated using the methods and equipment described in the

specification (i.e., pre-deposition of 3-HPA matrix in nanoliter volumes, resulting in a more crystalline and less amorphous matrix/analyte structure, upon analyte deposition), and then analyzed, that it was determined they could be successfully utilized for reproducible mass spectrometric analysis.

According to the analysis in the Declaration, there was no motivation to apply parameters for protein matrix substrates to 3-HPA substrates due to the significant differences between the matrix properties. Further, there was no reasonable expectation the 3-HPA substrates could be utilized successfully until they were manufactured and analyzed due to the distinct matrix properties.

Second, the Declaration clarifies the cited documents discussed methods of generating substrates having larger amounts of matrix and larger spot sizes. For example, the Declaration notes in paragraph 3:

...the spot diameter of the claimed substrates does not exceed 1.13 millimeters, whereas the spot diameter described in Nicola *et al.* and Vorm *et al.* is several millimeters (e.g., 10 millimeters) in diameter.

This observation can be taken in conjunction with the statement in Vorm that “the surface area of crystals should be very large” (page 3285, left column). Vorm states the surface area of the crystals should be “very large” so that only the outer surface of the crystal is redissolved by protein analyte (page 3285, left column). Thus, the person of ordinary skill in the art was discouraged from minimizing the area of the spots. As a result, there was no motivation to reduce the volume of matrix to that claimed, and the artisan had no reasonable expectation that reducing the matrix volume deposited on a substrate would successfully lead to reproducible MALDI mass spectra.

The motivation to reduce the matrix amount lacking in Vorm and Vestal also is lacking in Hayes. The disclosure in Hayes does not address mass spectrometry or MALDI analysis, fails to mention “matrix,” and is instead directed to methods for constructing diagnostic arrays having large numbers of probes. Hayes discussed an apparatus having multiple reservoirs for depositing different components on a substrate, not depositing matrix on substrates for MALDI MS. Hayes is silent with regard to providing nanoliter volumes of matrix for enhanced spot to spot reproducibility of MALDI mass spectra. A general discussion of repeatably depositing small volumes of probes on

a substrate provides no motivation to deposit small volumes of matrix on a substrate for MALDI MS analysis. Hayes focused on increasing probe density on chips, and did not suggest the desirability of reducing the volume of matrix deposited for MALDI MS. The artisan therefore had no reasonable expectation that reducing the volume of matrix on a substrate would successfully lead to reproducible MALDI mass spectra in view of Hayes. Hayes therefore provided no motivation to the artisan for depositing a 0.2 to 20 nanoliter volume of matrix as claimed when preparing a substrate for MALDI MS analysis.

Vorm also failed to teach or suggest depositing matrix in an array of spots. Vorm dispensed matrix in one spot on a probe tip. As none of the other documents in the cited combination teach or suggest depositing matrix, without analyte, in an array of spots, the claimed subject matter is not obvious over the combination of documents.

The statement in Vorm that “slight alterations” may be made to adapt the method to the system used (page 3283) is noted by Applicants. It is respectfully submitted, however, that generating substrates with significantly smaller spots of the distinct 3-HPA matrix is **not** a slight alteration given there was no motivation provided to utilize 3-HPA and there was no expectation that such substrates could be successfully utilized for mass spectrometric analysis.

The statement in Vorm that the discussed method “leads to the formation of a dense, flat, and thin film presumably consisting of very small crystals of matrix” (page 3282) also is noted. It is respectfully submitted, however, that these properties of the matrix components used in Vorm differ from the 3-HPA matrix properties, since 3-HPA yields crystals having amorphous morphologies that were not entirely conducive to mass spectrometric analysis (Declaration, paragraph 4). Accordingly, there was no motivation in Vorm to utilize the 3-HPA matrix as the latter exhibited undesirable properties.

Thus, the cited combination provided no motivation to reduce the volume of matrix deposited to 0.2 to 20 nanoliters, or deposit matrix in an array of spots. The cited combination also provided no motivation to spot 3-HPA on substrates in the volumes and sizes claimed due to distinct properties of this matrix. There also was no reasonable expectation the 3-HPA spotted substrates could be successfully utilized for mass spectrometric analysis. Accordingly, the pending claims are not *prima facie* obvious in view of the cited combination, and withdrawal of the rejection respectfully is requested.

Obviousness Rejection: Combination of Nicola, Li and Hayes

Claims 108-118, 120-123, 125, 132-141 and 143-146, were rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious by Nicola *et al.* in view of Li *et al.* and Hayes *et al.* The Office states Nicola teaches depositing microliter volumes of matrix on a MALDI MS substrate and applying protein analyte. The Office acknowledges that Nicola does not teach depositing drops of matrix in 0.2 to 20 nanoliter volumes or performing MALDI MS for nucleic acids. The Office quotes column 2, lines 49-55 of Hayes, stating that it would have been obvious to modify Nicola's MALDI MS analysis by using Hayes' method of depositing very small droplets (less than 1 nL) of the matrix material. The Office further states Li emphasizes reproducible spectra were obtained from 0.9 microliter deposits forming a thin matrix layer. The stated motivation to combine the documents is that Hayes teaches depositing smaller volumes increases reproducibility of the spots and MALDI MS was a well recognized method for analyzing DNA.

Applicants respectfully assert the rejection is moot in view of the amendment to claim 108. The claims are directed to methods of generating substrates that comprise spots of 3-HPA matrix. As in Vorm, this matrix is not discussed in Nicola or Li, which utilize matrix components suitable for protein analysis by mass spectrometry. In view of the Declaration submitted herewith, Nicola provided no motivation to prepare 3-HPA substrates and provided no reasonable expectation that the claimed substrates could be successfully utilized, as described in greater detail above.

Further, Nicola discusses matrix amounts and spot sizes far larger than what is claimed. The Declaration notes that Nicola spotted far larger amounts of matrix on substrates than claimed herein, which resulted in larger spot sizes. Nicola teaches using even larger amounts of matrix than Vorm. A quotation in Nicola is provided for clarity:

The areas of the substrates ranged from 25-100 mm²; thus, the matrix/acetone solution was deposited on the substrates in volumes of *ca.* 2.5-10 µL. This resulted in greater crystal thickness compared to previously published results which we consider essential for improvement in signal reproducibility (as previously reported, ¹³ much less matrix was used for the matrix crystal layer resulting in a much thinner layer).

Page 1166, left column, "Fast-evaporation sample preparation" section. The previous "report" in

reference 13 is the Vorm document addressed above. This passage clearly shows Nicola taught the crystal thickness should be increased over the thickness discussed in Vorm, and that a greater amount of matrix therefore should be spotted. Accordingly, Nicola taught away from using lower matrix amounts and lower volumes as is spotted in the claimed methods. And the artisan had no reasonable expectation that reducing the volume of 3-HPA matrix deposited on a chip would successfully lead to reproducible MALDI mass spectra.

Li discusses a probe tip having a 4 mm diameter covered by a thin layer of matrix. Thus, Li does not discuss an array of spots and the one spot discussed in Li has a larger diameter than any spots of the claimed substrates. Also, the spot size in Li (i.e., 4 mm) is larger than spots on substrates resulting from the claimed methods. The Office states Li discusses probe tips having a thin layer of matrix. This feature, however, is opposite to directions in Nicola to increase crystal thickness. In view of these diverging approaches, there was no motivation for combining Li and Nicola.

In conclusion, there was no motivation to combine Li with Nicola. The combination of Li and Nicola provided no motivation to prepare 3-HPA substrates, and there was no reasonable expectation the claimed substrates could be successfully utilized due to properties of the matrix. Hayes also provided no motivation to reduce the volume of matrix deposited in Nicola, provided no motivation to utilize 3-HPA, and therefore does not remedy the defects of Nicola and Li. Accordingly, the claimed substrates are **not** *prima facie* obvious in view of the cited combination and withdrawal of the rejection respectfully is requested.

Obviousness Rejection: Combination of Documents Discussed Above with Hancock

Claims 119, 124 and 126-131 were rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious by Nicola *et al.* and Li *et al.* in view of Hayes *et al.* or Vestal in view of Vorm *et al.* and Hayes *et al.*, further in view of Hancock. The Office stated Hancock disclosed the substrate materials specified by the rejected claims. The rejected claims directly or indirectly depend from amended claims 74 and 110 and include their limitations. The rejection therefore respectfully is traversed as Hancock fails to remedy the deficiencies described above. Hancock fails to provide any motivation for reducing the volume of matrix deposited on a substrate to the volumes claimed, provides no motivation for preparing substrates with 3-HPA matrix and does not provide a reasonable expectation for successfully utilizing the claimed substrates for mass spectrometry.

Thus, it is respectfully submitted that the subject matter of the rejected claims is **not** *prima facie* obvious over the cited combination and withdrawal of the rejection respectfully is requested.

CONCLUSIONS

Applicants respectfully submit the pending claims are in condition for allowance, and they earnestly solicit an early notice to such effect. That said, should any issues or questions remain, the Examiner is encouraged to telephone the undersigned at (858) 623-9470 so that they may be promptly resolved.

In the unlikely event the transmittal letter is separated from this document and the Office determines that an extension and/or other relief is required, Applicants petition for any required relief, including extensions of time, and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 503473**.

Respectfully submitted,

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By: _____

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